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ARIANI, KADE	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/562,248	HOLKER ET AL.	
	Examiner	Art Unit	
	Kade Ariani	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *9/21/09 and11/03/2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *32-84* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *32-84* is/are rejected.

7)☒ Claim(s) *55,61,65 and 69* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *2/13/2007*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The preliminary amendment filed on June 29, 2006, has been received.

Claims 1-31 have been canceled.

New claims 32-84 have been added.

Claims 32-84 are pending in this application and were examined on their merits.

Election/Restrictions

Applicant's request for reconsideration is considered, therefore the restriction requirement is hereby withdrawn.

Claim Objection

Claims 55, 61, 65, and 69 are objected to because of the following informalities:

In claim 55 (lines 3) delete “processes” and insert –processing-- in its place.

In claim 61 (line 2) delete "tubigensis" and insert --tubingensis-- in its place.

In claim 65 (line 2) delete "tugibensis" and insert --tubingensis-- in its place.

In claim 69 (line 2) delete "tubigensis" and insert --tubingensis-- in its place.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 50-54 and 73 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 50-54 recite the limitation "wherein the continuously produced enzyme/substrate/fungus mixtures". There is insufficient antecedent basis for this limitation in these claims, since claim 32 does not recite “continuously produced enzyme/substrate/fungus mixtures”.

Claim 73 (line 2, part a) recites the limitation “said preinduced mixtures…”. There is insufficient antecedent basis for this limitation in these claims, since claim 32 does not recite “preinduced mixtures”.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless

> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 32-38, 41-45, 54, 55, 56 and 83 are rejected under 35 U.S.C. 102(b) as being anticipated by Gutierrez-Correa et al. (in IDS, Bioresource Technology, 1999, Vol. 68, p.173-178) as evidenced by "NCBI Taxonomy search results for *A. niger*" and by Durand, A., (Biochemical Engineering Journal, March 2003, Vol. 13, p.113-125).

Gutierrez-Correa et al. disclose a method for solid substrate fermentation comprising culturing a mixed culture of *Trichoderma reesei* and *Aspergillus niger* (at least two microorganism one fungi form Aspergillus sp. and one fungi *Trichoderma reesei*) in a flask (a solid phase laboratory-scale bioreactor) on agricultural residue sugar cane bagasse (a waste material) for the production of cellulolytic enzymes, cocktail of hydrolases (cellulase, endoglucanase, and β-glucosidase), bagasse was supplemented with soymeal (a natural raw material) and fermented at 80% moisture content and at 30°C (under appropriate selection pressure and based on an optimal inoculation procedure) for a defined culturing time (36 and 48 hours), co-culturing increased enzyme production, the substrate was autoclaved (a semi-sterile method), and soymeal supplementation increased biomass production and increased xylanase production (an inducer substrate or inductive substrate) (Abstract and p.174 1st column 2.1. paragraph lines 5-6 and 2nd column 2.3. the whole paragraph, and p.176 1st column 2nd paragraph lines 1-2). It must be noted that *Aspergillus niger* is an ascomycetes fungus (see NCBI Taxonomy search result for *A. niger*). Moreover, according to Durand a flask is a laboratory-scale bioreactor (see p. 114 1st column 3rd paragraphs 2. lines 1-2 and 2.1. lines 1-3). Gutierrez-Correa et al. disclose stock cultures were maintained on agar slants (a pre-culture of microorganism adapted to solid substrates) and spores

were added to shake flasks containing basal medium supplemented with bagasse (to produce the mycelial inoculum used in SSF) (culture was run through an inductive pre-culture) (p.174 1st column, 2.2. and 2.2.- continued on 2nd column). Gutierrez-Correa et al. disclose the nutritionally poor substrate (sugar cane bagasse) was more suitable for cellulase production in mixed culturing (p.176 1st column 1st paragraph lines 4-6). Gutierrez-Correa et al. further disclose shaking the contents of the solid fermentation flasks with water, filtering, and the liquid part (the filtrate) was used for enzyme determination (an enzyme mixture obtained by the method), while the solids collected for dry matter and biomass determination (p.174 2nd column paragraph 2.4.).

Gutierrez-Correa et al. therefore clearly anticipate the claimed method and enzyme mixture.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 32-45, 50-58, 71-80, 83 and 84 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gutierrez-Correa et al. (in IDS, Bioresource Technology, 1999, Vol. 68, p.173-178) and "NCBI Taxonomy search results for *A. niger*" and Durand, A.,

(Biochemical Engineering Journal, March 2003, Vol. 13, p.113-125) in view of Tengerdy et al. (Biochemical Engineering Journal, March 2003, Vol. 13, p.169-179) and further in view of "WO 02/10099 A2, Abstract" and of Raimbault, M. (EJB Electronic Journal of Biotechnology, 1998, Vol. 1, No.3, p.174-188).

Claims 32-38, 41-45, 54-56 and 83 are drawn to a semi-sterile method of producing a defined enzyme mixture comprising, a) contacting a mixed culture of microorganisms in a solid-phase bioreactor with one or more substrates, b) culturing the mixed culture under an appropriate culturing parameters chosen from moisture content (pH value, temperature, oxygen, .. and, a specific induction by the target substrate, inducer substrate or combinations thereof, and inhibition with appropriate inhibitors) for a defined culturing time, the inoculating mixed culture is obtained by culturing a preculture of mixed microorganism adapted to solid substrates, inoculating mixed culture is a culture which has been run through an inductive preculture and one culture operated under selection pressure, the substrates are selected from raw materials, at least two microorganisms are employed for producing mixed cultures, at least one microorganisms is a fungi, one microorganism (fungi) is an ascomycetes, at least one fungi from Aspergillus sp., *Aspergillus niger*, the mixed cultures are suitable for the continuous production of specific hydrolase cocktail, the hydrolase cocktails are suitable for use in the processing of the residual materials, and an enzyme mixture.

Gutierrez-Correa et al. teach a method for solid substrate fermentation comprising culturing a mixed culture of *Trichoderma reesei* and *Aspergillus niger* (at least two microorganism one fungi form Aspergillus sp., and one fungi of species

Trichoderma reesei) on agricultural residue sugar cane bagasse (waste material) for the production of cellulolytic enzymes hydrolase cocktails (cellulase, endoglucanase, and β-glucosidase) bagasse was supplemented with soymeal (a natural raw material) and fermented at 80% moisture content and at 30°C (under appropriate selection pressure and based on an optimal inoculation procedure) for a defined culturing time (36 and 48 hours), co-culturing increased enzyme production, the substrate was autoclaved (a semi-sterile method), and soymeal supplementation increased biomass production and increased xylanase production (an inducer substrate or inductive substrate) (Abstract and p.174 1st column 2.1. paragraph lines 5-6 and 2nd column 2.3. paragraph, and p.176 1st column 2nd paragraph lines 1-2). It must be noted that *Aspergillus niger* is an ascomycetes fungus (see NCBI Taxonomy search result for *A. niger*). Gutierrez-Correa et al. teach stock cultures were maintained on agar slants (a pre-culture of microorganism adapted to solid substrates) and spores were added to shake flasks containing basal medium supplemented with bagasse (to produce the mycelial inoculum used in SSF) (culture was run through an inductive pre-culture) (p.174 1st column, 2.2. and 2.2.- continued on 2nd column). Gutierrez-Correa et al. teach the nutritionally poor substrate (sugar cane bagasse) was more suitable for cellulase production in mixed culturing (p.176 1st column 1st paragraph lines 4-6). Gutierrez-Correa et al. further teach bioconversion of lingocellulosic biomass the most abundant organic raw material in the world, into ethanol is feasible using an efficient enzyme system and reducing the cost of bioconversion (of lingocellulosic materials into ethanol) will stimulate new markets for the agriculture sector, but due to its complexity lingocellulosic bioconversion requires

the action of multiple enzymes (for complete hydrolysis of cellulose), however a critical point in the enzyme-based technologies is the production cost of an efficient enzyme system, complete hydrolysis of cellulose requires the action of the cellulase system containing cellobiohydrolase, endoglucanase and β-glucosidase, both fungi and bacteria can degrade lingocellulose, and both can produce lingocellolytic enzymes by fermentation in solid substrate systems (p.173 "1. Introduction" 1st column 1st and 2nd paragraphs and 2nd column 1st paragraph and 2nd paragraph lines 1-3). Gutierrez-Correa et al. teach symbiotic associations in mixed cultures not only overcompensate for nutrient limitations in the substrate, this gives a solid economic advantage to mixed culturing over single culturing for enzyme production on limited agricultural waste products, saving costs of expensive organic supplements (p.176 2nd column 3rd paragraph lines 4-5 continued on page 177 1st column 2nd paragraph lines 1-5). Gutierrez-Correa et al. further teach shaking the contents of the solid fermentation flasks with water, then filtering, and the liquid part (the filtrate) was used for enzyme determination (an enzyme mixture obtained by the method, while the solids collected for dry matter and biomass determination (p.174 2nd column paragraph 2.4.). Gutierrez-Correa et al. also teach mixed culture fermentations are used in anaerobic digestion of organic matter (producing enzymes which are suitable for fermentation under anaerobic conditions) and product and process-specific mixed culture fermentations have been used for biodelignifiaction and enzyme production (p.174 1st column 1st paragraph lines 1-10).

Gutierrez-Correa et al. do not teach (claim 84) a bioreactor comprising a fermentation module which comprises regulation means to adjust a fermentation environment, a feeding means being connected to the fermentation module, and induction module for adding reagents to the fermentation media, a harvesting module comprising outlet means, and a conveying means to convey the media from the fermentation module through the induction module to the harvesting module, (claims 74-77) wherein the solid phase cultures are performed a screw reactor, screw conveying, and solid phase cultures are in cascade form, (claims 50-58) the method is performed in a continuous manner, the continuously produced enzyme/substrate/fungus mixtures are suitable to be used as such, the continuously produced enzyme/substrate/fungus mixtures are suitable after separation of the substrate/fungus to obtain a liquid enzyme cocktail, the continuously produced enzyme/substrate/fungus mixtures are suitable to be used for the saccharification of natural polysaccharides, the continuously produced enzyme/substrate/fungus mixtures are substituted by enzymes which are prepared by means of other methods, (claims 39 & 40) the moisture content is used for controlling the selection pressure by the addition of water and its removal by means of temperature and suction, the water activity is between 0.85 and 0.99, (claims 71-73) the pre-induced mixtures of microorganism and enzymes are directly supplied to the downstream processes, the pre-induced mixtures of microorganism and enzymes are transferred to another solid state process operation in which the whole substrate which is to be fermented later is utilized for producing enzymes and at least partially hydrolyzed, and preinduced mixtures of white rot fungi (claims 78-80) the method further comprising

conservation of the obtained mixed culture by decreasing the water activity during the fermentation process, the water activity is decreased by air flow through the substrate or by a final drying step, the final drying step is in a fluidized bed.

However, Tengerdy et al. teach a solid state (solid-phase) bioreactor, a continuous treatment system and a screw-type solid state bioreactor with screw conveyors (conveying means) (see p.175, Figure 4. Legend and p.174, 2nd column 3rd paragraph lines 5-9). Tengerdy et al. also teach a fluidized bed reactor, reactor with six sample ports, pump, gas flow, pH and temperature control devices (regulation means to adjust a fermentation environment), and feed reservoir (feeding means, module for adding reagents to the fermentation media and outlet means) (p.171 see Figure 1. Legend). Tengerdy et al. teach in fluidized bed reactor the particles (fungi spores + corncob reached a desired thickness) were drained and used as starters, or stored after sterile air drying to provide a finished starter (air flow through the substrate or by a final drying step and conservation of the obtained culture) (p.170 2nd column 4th paragraph). Tengerdy et al. teach the advantage of fluidized bed reactor is that spores are rapidly germinated making possible easy dosing and fast start in the bioreactor (p.170 2nd column 4th paragraph lines 3-4). Tengerdy et al. teach for lingocellulose SSF, enzymes must be pre-induced for a quick start of lingocellulose breakdown and fungal growth (p.170 2nd column 2nd paragraph lines 13-15). Tengerdy et al. teach enzyme production by SSF may be a good choice for many agrobiotechnological applications where the crude enzyme source can be directly used in a process (e.g. biofuel, biopulping, bioleaching) (enzyme mixtures directly supplied to the downstream processes) (p.172

2nd column 1st paragraph 15-18). Tengerdy et al. teach process of pre-bioleaching where substrate specific filamentous fungi and actinomycetes were grown on eucalyptus and bagasse pulps by SSF and fermented substrates, containing predominantly xylanases and only traces of cellulases, where used for bioleaching (using enzyme mixtures are transferred to another solid state solid process operation in which the whole substrate which is to be fermented later is selectively utilized for producing enzymes and partially hydrolyzed) (p. 174 2nd column 2nd paragraph lines 3-9). Tengerdy et al. further teach in a SSF reactor the most critical parameters are moisture, oxygen supply and temperature control, and computer controlled on-line evaporative moisture and temperature control in a bioreactor, and controlling temperature by forced evaporation (the moisture content is used for controlling the selection pressure by the addition of water and its removal by means of temperature and suction) (p.172 1st column 1st paragraph lines 8-12). Tengerdy et al. teach lingocellulosic biomass and wastes represent a vast alternative source for ethanol production, application of the SSF technology promise cost reduction and higher hydrolytic efficiency in bioethanol process (p.172 2nd column 3rd paragraph lines 1-3, end paragraph) (It must be note that bioethanol production is the simultaneous saccharification and co-fermentation process) (p. 173 1st column 2nd paragraph lines 3-5). Tengerdy et al. teach the cost of commercial enzymes prohibit their application for bioethanol production or any large-scale agrobiotechnological application (p.173 1st column 3rd paragraph lines 1-2 and 13-16). Tengerdy et al. teach the direct in situ applicability of enzymes produced by SSF technology (p.174 1st column end

paragraph). Tengerdy et al. teach a lingocellulosic agricultural residue, fermented with lingocellulolytic and other fungi in single or mixed culture SSF may yield a directly applicable feed supplement (p.173 2nd column 3rd paragraph lines 7-11). Tengerdy et al. teach white rot fungi are the most efficient decomposers of wood and other natural lingocellulose (p.169 2nd column paragraph 2., lines 2-4). Tengerdy et al. teach since nutrient availability is more restricted in natural solid substrates fungi are able to develop more efficient enzyme systems for host cell degradation than in liquid cultures and this translate to more efficient hydrolysis of the substrates in a bioreactor (p.170 1st column 1st paragraph lines 4-7).

"WO 02/10099 A2" teach a bioreactor for solid state fermentation with parallel (cascade form) reaction batches suitable for optimizing and upscaling fermentation reactions (see Abstract).

Raimbault teaches the fungi used in SSF processes have minimum growth Aw values between 0.8 and 0.9 (water activity is between 0.85 and 0.99) (p.184 2nd column 2nd paragraph lines 12-14).

Therefore, in view of the above teachings, a person of ordinary skill in the art at the time the invention was made, would have been motivated to use the method as taught by Gutierrez-Correa et al. to inoculate a mixed culture of white rot fungi in a solid phase bioreactor using water activity between 0.85 and 0.99, according to the teachings of Tengerdy et al. and Raimbault., with a reasonable expectation of success to provide a semi-sterile method of producing a defined enzyme mixture and an enzyme mixture. Because Raimbault teaches the fungi used in SSF processes have minimum growth Aw

values between 0.8 and 0.9, and because Tengerdy et al. teach white rot fungi are the most efficient decomposers of natural lingocellulose. The motivation as taught by Tengerdy et al. would be application of the SSF technology promise cost reduction and higher hydrolytic efficiency and enzyme production by SSF is good choice for many agrobiotechnological applications where the crude enzyme source can be directly used in a process. Moreover, a person of ordinary skill in the art at the time the invention was made recognizing different applications would require different design of solid state bioreactor, would have been motivated to use the method as taught by Gutierrez-Correa et al. to inoculate a mixed culture of fungi in a screw-type solid state bioreactor with screw conveyors, a fluidized bed reactor solid phase bioreactor according to the teachings of Tengerdy et al. and WO 02/10099 A2 with a reasonable expectation of success to provide a semi-sterile method of producing a defined enzyme mixture and an enzyme mixture. Because Tengerdy et al. teach using a screw-type solid state bioreactor and continuous treatment system with screw conveyors to apply SST technology in composting, and fungal SSF in a fluidized bed reactor to produce mixed fungal biomass (starter). The motivation as taught by Tengerdy et al. would be the advantage of fluidized bed reactor is easy dosing and fast start in the bioreactor.

Claims 32-38, 41-48, 54-56, and 83 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gutierrez-Correa et al. (inn IDS, Bioresource Technology, 1999, Vol. 68, p.173-178) and "NCBI Taxonomy search results for *A. niger*" and Durand, A., (Biochemical Engineering Journal, March 2003, Vol. 13, p.113-125) in view of Bradley

et al. (US 6,485,952 B1) and further in view of Pandey et al. (Process Biochemistry, 2000, Vol. 53, p.1153-1169) and NCBI Taxonomy search for *Streptomyces clavuligerus*.

As mentioned immediately above, Gutierrez-Correa et al. teach the limitations of claims 32-38, 41-45, 54-56, and 83.

Gutierrez-Correa et al. do not teach the fungi from Trametes sp., at least one bacterium of the order actinomycetes, at least one bacterium from Streptomyces sp. However, Bradley et al. teach solid state fermentation by culturing fungi of Trametes sp. (*Trametes versicolor*) using sugar beet pulp (column 7 Example 4, lines 39-45). Bradley et al. teach sugar beet pulp substrate is capable of inducing the production of enzymes (peroxidases, manganese peroxidase, oxidases, and laccases) and sustaining the growth of variety of white rot fungi (column 5 lines 45-47).

Moreover, Pandey et al. (2000) teach using bacteria of Streptomyces sp. (*Streptomyces clavuligerus* order of Actinomtycetes) in SSF to produce a desired metabolite (a bioactive compound) (p.1154 Table 1. Production of bioactive compounds in SSF, source # 9, also see NCBI Taxonomy search result for *Streptomyces clavuligerus*).

Therefore a person of ordinary skill in the art at the time the invention was made, would have been motivated to use the method as taught by Gutierrez-Correa et al. according to the teachings of Bradley et al. by using a fungi from Trametes sp. in the mixed culture and sugar beet pulp with a reasonable expectation of success in providing a semi-sterile culture method for producing an enzyme mixture by SSF on sugar beet pulp. Because Bradley et al. teach production of enzymes mixture by fungi of Trametes

sp. in solid state fermentation using sugar beet pulp. Moreover, a person of ordinary skill in the art a the time the invention was made, would have been motivated to use the method as taught by Gutierrez-Correa et al. by using one bacterium from Streptomyces sp. in a mixed culture according to the teachings of Pandey et al. with a reasonable expectation of success to provide a semi-sterile culture method for producing useful metabolite(s). Because Pandey et al. teach using bacteria of Streptomyces sp. in SSF to produce metabolite (a bioactive compound).

Claims 32-38, 41-45, 54-56, 59-70 and 83 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gutierrez-Correa et al. (in IDS, Bioresource Technology, 1999, Vol. 68, p.173-178) and "NCBI Taxonomy search results for *A. niger*" and Durand, A., (Biochemical Engineering Journal, March 2003, Vol. 13, p.113-125) in view of De Vries et al. (Applied and Environmental Microbiology, 1997, Vol. 63, No. 12, p.4638-4644) and of El-batal (Food Research International, 2001, Vol. 34, p.715-720) and of Viveros et al (J. Agric. Food Chem., 2000, Vol. 48, p.4009-4013) and of Malherbe et al. (Re/View in Environmental Science & Bio/Technology, 2002, Vol. 1, p.105-114) and of Mach et al. (Applied and Environmental Microbiology, 1999, Vol. 65, No.5, p.1858-1863) and of Tengerdy et al. (Biochemical Engineering Journal, March 2003, Vol. 13, p.169-179) and of Chiou et al. (Asian-australasian journal of animal science, 2002, Vol. 15, No.3, Abstract) and further in view of Raimbault, M. (EJB Electronic Journal of Biotechnology, 1998, Vol. 1, No.3, p.174-188).

As mentioned immediately above, Gutierrez-Correa et al. teach the limitations of claims 32-38, 41-45, 54-56, and 83.

Gutierrez-Correa et al. do not teach (claims 59-62) the enzyme mixture is a suitable for the enzymatic extraction of sugar beet chips or a polysaccharide-containing material, the inducer is a rape extraction material, one of the two fungi in the mixed culture is *A. tubingensis*, during the culture process *Neurospora intermedia* is added to the mixed culture, and the water activity is reduced to about 0.96, (claims 63-66) an enzyme mixture suitable for the enzymatic extraction of grass silage, the inducer is rape extraction material, microorganisms are *A. niger*, *A. tubingensis,* and *Neurospora intermedia*, during the culture *Trichoderma atroviridae* and grass silage as substrate are added to the mixed culture and the water activity is raised to about 0.99. (claims 67-70) producing an enzyme mixture suitable for the enzymatic extraction of corn silage, wherein the inducer is a rape extraction material, the microorganisms are *A. niger*, *A. tubingensis,* and *Neurospora intermedia*, during the culture *A. oryzae* and corn silage as substrate are added to the culture and the water activity is raised to about 0.99. However, De Vries et al. teach cinnamic acids bound to polysaccharides in cell walls of plants decrease the cell wall biodegradability, *A. tubingensis* produce a specific esterase enzyme (ferulic acid esterase), with the ability to degrade cell wall through hydrolysis and release of ferulic acid from the sugar beet pectin (it must be noted that ferulic acid is a cinnamic acid), and the enzyme can be induced by the growth on sugar beet pectin (Abstract and Introduction 1st paragraph lines 1-8). De Vries et al. teach A.

niger also produce an esterase (cinnamoyl esterase) with the ability to release ferulic acid from sugar beet pectin (Introduction 1st column 1st paragraph lines 12-14).

Moreover, O'Toole teaches *Neurospora intermedia* is suitable to digest insoluble fibers in solid state fermentation of a waste material mostly made of lignin, cellulose and hemicellulose (okara the residue left from ground soy beans) (p.366 1st column 4th paragraph lines 20-26, Abstract, and p.363 Introduction 1st paragraph lines 1-3).

Furthermore, El-batal et al. teach production of phytase (enzyme) by *A. niger* when grown on rapeseed meal (rape extraction material) during SSF, and optimum moisture content of the media was 60% (see Abstract). El-batal et al. also teach the optimum amount of water varies and must be determined for each system and microorganism (p.717 1st column 2nd paragraph lines 1-2). El-batal et al. teach the presence of anti-nutritional compounds polyphenols and phytic acid in the rape seed (Introduction 1st column end paragraph lines 1-2 &5-6). It must be noted that at the time the invention was made it was well known in the art that sugar beet pulp contains phytate (see Viveros et al. p.4010 2nd column 4th paragraph lines 1-4).

Malherbe et al. teach in grasses ferulic acids associated with lignin and shield hemicellulose from direct enzymatic hydrolysis (p.106 2nd column 4th paragraph 4-5 and 13-15). Malherbe et al. further teach grasses are more susceptible to actinomycetes attack than wood (p.108 2nd column paragraph 3.3.1. and p.109 1st column 1st paragraph line 1), and white rot fungi selectively degrade lignin (p.111 1st column 2nd paragraph lines 1-3). Malherbe et al. the key enzymes of lignin degradation by white-rot fungi are phenol oxidases (p.107 2nd column 2nd paragraph lines 1-3). Malherbe et al.

also teach xylan is the most common hemicellulose component of grass and wood (p.106 1st column 3rd paragraph lines 17-19).

Mach et al. teach *Trichoderma atroviridae* is a mycroparasitic biocontrol agent (due to its ability to produce chitinolytic enzymes) (Abstract and Introduction 1st column 3rd paragraph lines 1-6).

Tengerdy et al. teach crude cellulolytic enzymes can be produced on corn silage by SSF and used to improve ensiling efficiency (p.176 2nd column 2nd paragraph lines 11-14).

Chiou et al. teach adding *A. oryzae* fermentation extract (AFE) to the ensiling corn silage and its effect on the performance of lactating cows fed corn silage, and inclusion of *A. oryzae* fermentation extract (AFE) in corn silage significantly improved the dry matter intake by dairy cows, (see Abstract).

Furthermore, Raimbault teaches the fungi used in SSF processes have minimum growth Aw values between 0.8 and 0.9 (water activity is between 0.85 and 0.99) (p.184 2nd column 2nd paragraph lines 12-14). Raimbault teaches the optimum water activity (Aw) for growth of a number of fungi used in SSF processes was at least 0.96 (p.184 2nd column end paragraph continued on 2nd column end paragraph). Therefore, a person of ordinary skill in the art at the time the invention was made would have realized that water activity is a result-effective variable and would have been optimized by routine experimentation.

Therefore, a person of ordinary skill in the art at the time the invention was made, knowing that *A. tubingensis* produce a specific esterase enzyme with the ability to

degrade cell wall and release of ferulic acid from the sugar beet pectin, *Neurospora intermedia* ability to digest insoluble fibers in solid state fermentation of a waste material, and induction of phytase (enzyme) by *A. niger* when grown on rapeseed meal during SSF, would have been motivated to use the method as taught by Gutierrez-Correa et al. by using a mixed fungi culture comprising *A. tubingensis, Neurospora intermedia,* and *A. niger* using rape extraction material as an inducer according to the teachings of De Vries et al., Pandit et al., O'Toole, and El-batal et al., with a reasonable expectation of success in providing a semi-sterile culture method for producing an enzyme mixture (including phytase) suitable for degradation or hydrolysis of sugar beet chips (since sugar beet pulp contains phytate).

Moreover, a person of ordinary skill in the art at the time the invention was made, knowing that the in grasses ferulic acids associated with lignin and shield hemicellulose from direct enzymatic hydrolysis and phenol oxidases are key enzymes of lignin degradation, and the presence of polyphenols in the rape seed to induce the production of phenol oxidases, would have been motivated to use the method as taught by Gutierrez-Correa et al. by using a mixed fungi culture comprising *A. tubingensis, A. niger*, and *Neurospora intermedia* on grass silage, using inducer rapeseed meal during SSF, and adding *Trichoderma atroviridae* according to the teachings of De Vries et al., Pandit et al., O'Toole, and El-batal et al., Malherbe et al. and Mach et al., with a reasonable expectation of success in providing a semi-sterile culture method for producing an enzyme mixture suitable for degradation of grass silage and culturing *Trichoderma atroviridae.*

Furthermore, a person of ordinary skill in the art at the time the invention was made, knowing cellulolytic enzymes can be produced on corn silage by SSF and used to improve ensiling efficiency, would have been motivated to use the method as taught by Gutierrez-Correa et al. by using a mixed fungi culture comprising *A. tubingensis, A. niger*, and *Neurospora intermedia* on corn silage, rapeseed meal during SSF, and adding *A. oryzae* according to the teachings of De Vries et al., Pandit et al., O'Toole, Tengerdy et al. and Chiou et al., with a reasonable expectation of success in providing a semi-sterile culture method for producing an enzyme mixture suitable for degradation of corn silage. The motivation as taught by Chiou et al. would be the inclusion of *A. oryzae* fermentation extract (AFE) in corn silage significantly improved the dry matter intake by dairy cows, and as taught by Tengerdy et al. would be to improve ensiling efficiency.

Accordingly, determination of the water activity to be used in the SSF method as taught by Gutierrez-Correa et al. would have been a matter of routine optimization to a person of ordinary skill in the art, said person recognizing that Raimbault teaches the optimum water activity for growth of fungi used in SSF processes of at least 0.96, and because El-batal et al. teach the optimum amount of water activity must be determined for each system.

Claims 32-38, 41-45, 54-56, and 81-83 are rejected under 35 U.S.C. 103(a) as being unpatentable over Gutierrez-Correa et al. (Bioresource Technology, 1999, Vol. 68, p.173-178) and "NCBI Taxonomy search results for *A. niger*" and Durand, A., (Biochemical Engineering Journal, 2003, Vol. 13, p.113-125) in view of Palit et al.

(Brazilian Archives of Biology and Technology, 2001, Vol. 44, No.1, p.107-111) and further in view of Pandey et al. (Current Science, 1999, Vol. 77, No.1, p.149-162, 22 pages in "pdf").

As mentioned immediately above, Gutierrez-Correa et al. teach the limitations of claims 32-38, 41-45, 54-56, and 83. Moreover, as mentioned immediately above, Gutierrez-Correa et al. teach shaking the contents of the solid fermentation flasks with water, then filtering, and the liquid part (the filtrate) was used for enzyme determination (p.174 2nd column paragraph 2.4.).

Gutierrez-Correa et al. do not teach leaching of the produced enzyme mixture is carried out for 30 minutes to 2 hours and wherein the filtrate is further used as solvent for additional leaching cycles to obtain highly concentrated enzyme slurry, and wherein the filtrate is used as a solvent for up to 10 additional leaching cycles. However, Palit et al. teach leaching an enzyme form SSF, using water (p.108 1st column Results & Discussion, last paragraph lines 4-5), repeated washing using agitation (Abstract), the time period was varied from 30 minutes to 270 minutes (p.109 2nd column 2nd lines 5-6) and recirculation (the filtrate is further used as solvent for additional leaching cycles) (p.110 1st column 1st paragraph lines 1-4). Palit et al. also teach that extraction (leaching) parameters including type of solvent, soaking time, physical state of leaching, and number of washes need to optimized (p.108 1st column 2nd paragraph).

Pandey et al. (1999) teach the recovery of the enzyme form the fermented matter during SSF is an important factor that effects the cost-effectiveness of the overall process (p. 15 of the PDF, 4th paragraph).

Therefore, a person of ordinary skill in the art at the time the invention was made, recognizing the recovery of the enzyme form the fermented matter during SSF is an important factor that effects the cost-effectiveness of the overall process, would have been motivated to apply the teachings of Pandit et al. with a reasonable expectation of success in optimizing the leaching of the enzyme mixture produced in the semisterile culture method for producing a define enzyme mixture according to the teachings of Gutierrez-Correa et al.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kade Ariani whose telephone number is (571) 272-6083. The examiner can normally be reached on IFP.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on (571) 272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Kade Ariani/
Examiner, Art Unit 1651